

# Alternating *syn-anti* bacteriochlorophylls form concentric helical nanotubes in chlorosomes

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Chlorosomes are the largest and most efficient light-harvesting antennae found in nature, and they are constructed from hundreds of thousands of self-assembled bacteriochlorophyll (BChl) *c*, *d*, or *e* pigments. Because they form very large and compositionally heterogeneous organelles, they had been the only photosynthetic antenna system for which no detailed structural information was available. In our approach, the structure of a member of the chlorosome class was determined and compared with the wild type (WT) to resolve how the biological light-harvesting function of the chlorosome is established. By constructing a triple mutant, the heterogeneous BChl *c* pigment composition of chlorosomes of the green sulfur bacteria *Chlorobaculum tepidum* was simplified to nearly homogeneous BChl *d*. Computational integration of two different bioimaging techniques, solid-state NMR and cryoEM, revealed an undescribed *syn-anti* stacking mode and showed how ligated BChl *c* and *d* self-assemble into coaxial cylinders to form tubular-shaped elements. A close packing of BChls via  $\pi$ - $\pi$  stacking and helical H-bonding networks present in both the mutant and in the WT forms the basis for ultrafast, long-distance transmission of excitation energy. The structural framework is robust and can accommodate extensive chemical heterogeneity in the BChl side chains for adaptive optimization of the light-harvesting functionality in low-light environments. In addition, *syn-anti* BChl stacks form sheets that allow for strong exciton overlap in two dimensions enabling triplet exciton formation for efficient photoprotection.

cryoEM | light-harvesting | photosynthesis | solid-state NMR | supramolecular aggregates

Chlorosomes are the principal light-harvesting apparatus of green sulfur bacteria, some green filamentous anoxygenic phototrophs, and the recently discovered aerobic anoxygenic phototroph *Candidatus Chloracidobacterium thermophilum* (1, 2). They are irregular, elongated sacs containing self-assembled BChls. They differ from other antenna complexes by their very large size ( $\approx 150 \times 60$  nm) and the absence of a protein matrix to support the photosynthetic pigments (1, 3, 4). Up to 250,000 BChls are organized into supramolecular light-harvesting devices with strongly red-shifted absorption bands and extremely large exciton diffusion lengths that cover up to 200 BChl monomers (5). Chlorosomes efficiently harvest light and are remarkably stable against photodegradation because of self-protection of the BChls from singlet oxygen (1, 6). They can form under extreme environmental conditions and allow for photosynthesis at extraordinarily low light intensities by ultrarapid transfer of energy into photosynthetic reaction centers (5, 7, 8). The unique optical and excitonic properties of chlorosomes are unknown for any other material, and chlorosomes may thus provide a basis for the rational design of biomimetic, self-assembling, and self-repairing structures for artificial photosynthesis and molecular electronics (9–11).

Chlorosomes are assembled from BChl *c*, *d*, or *e* and contain naturally heterogeneous molecular mixtures of BChls with different side chain modifications and stereochemistry (Fig. 1). In particular, chlorosomes of the green sulfur bacterium *Chlo-*

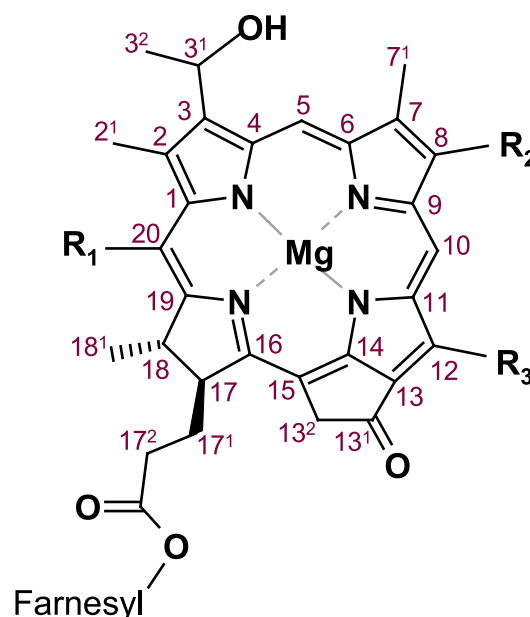


Fig. 1. BChl homologues for *C. tepidum*. In the WT,  $R_1$  = Me;  $R_2$  = Et, *n*-Pr, or *i*-Bu;  $R_3$  = Me, or Et. In the *bchQRU* mutant  $R_1$  = H;  $R_2$  = Et;  $R_3$  = Me.

*robaculum tepidum* contain a complex mixture of BChl *c* molecules with variable degrees of methylation at carbons C-8<sup>2</sup> and C-12<sup>1</sup> (Fig. 1), variations in the esterifying alcohol side chain of the C-17 carboxyl group, and both *R*- and *S*-chirality at the C-3<sup>1</sup> carbon (1, 12). Because a heterogeneous structure cannot be determined by crystallographic methods, chlorosomes are the only class of antennae for which no structural information is currently available. Conflicting models have been derived from spectroscopic and electron microscopic assessments (13–16) and from studies with chemical analogs (17).

Recent advances in understanding the biosynthesis of BChl *c* have led to a well-characterized *bchQ bchR bchU* (hereafter *bchQRU*) *C. tepidum* triple mutant (12). This mutant synthesizes well-defined, extended chlorosomes that contain >95% 17<sup>2</sup>-farnesyl-*R*-[8-ethyl,12-methyl]BChl *d* (Fig. 1) and form regularly packed, tubular-shaped elements with a diameter >10 nm that are interconnected via curved sheets (16, 18). The

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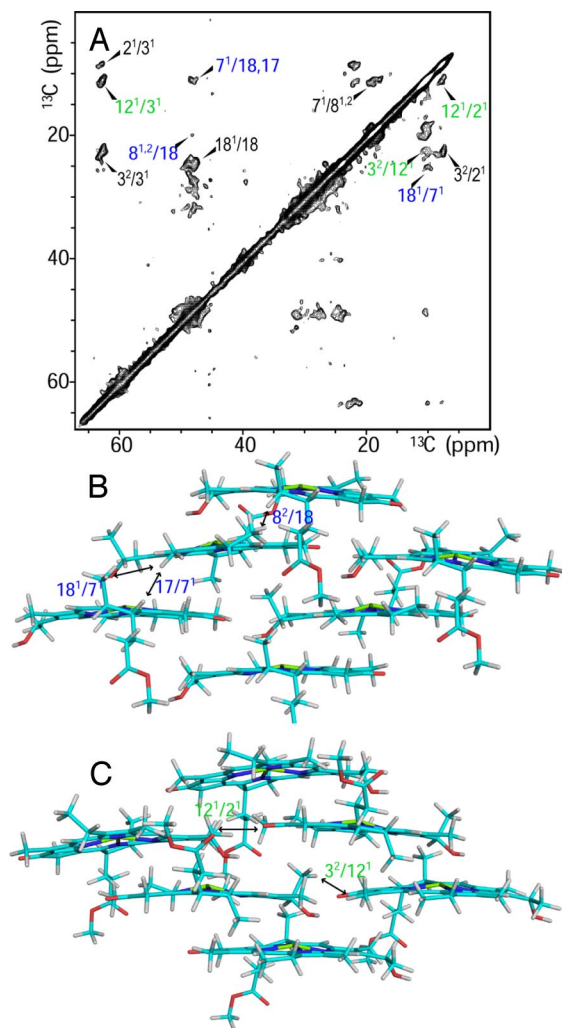
The authors declare no conflict of interest.

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**Fig. 2.** Distance constraints from proton-mediated correlation spectroscopy. (A)  $^{13}\text{C}$ - $^{13}\text{C}$  CHHC dataset of *bchQRU* chlorosomes, recorded at 17.6 T with 13 kHz spinning and 250  $\mu\text{s}$  spin diffusion. Correlations labeled in blue are intermolecular intrastack, in green are intermolecular interstack, and black are intramolecular, respectively. (B) Two *syn-anti* stacks with three BChl *d* molecules each (farnesyl tails are replaced by methyl groups). The arrows indicate the side chains between which cross-peaks are seen. Intermolecular intrastack correlations are labeled. (C) View of *B* from the opposite side with the interstack cross-peaks indicated.

**Table 1.** List of intermolecular cross-peaks observed between the BChl *d* molecules of chlorosomes of the *bchQRU* mutant in the CHHC spectrum for a mixing time of 250  $\mu$ s

CHHC contact	Shortest $^1\text{H}-^1\text{H}$ distance, Å
$7^1/17$	2.32
$7^1/18^1$	2.67
$8^2/18$	1.84
$2^1/12^1$	2.80
$3^1/12^1$	2.64
$3^2/12^1$	2.78

The corresponding shortest  $^1\text{H}$ – $^1\text{H}$  distances taken from an optimized *syn-anti* stack aggregate of six stacks, each stack comprising 12 *syn-anti* coordinated BChl *d* molecules are listed. Optimization was done with molecular mechanics in Hyperchem 7.

## Results

The NMR assignments of the  $^{13}\text{C}$  and  $^1\text{H}$  signals of BChl *d* molecules in chlorosomes from the *bchQRU* mutant were obtained from 2D  $^{13}\text{C}$ - $^{13}\text{C}$  and  $^1\text{H}$ - $^{13}\text{C}$  magic-angle spinning (MAS), solid-state NMR dipolar correlation spectra [supporting information (SI) Figs. S1 and S2 and Tables S1 and S2] collected from  $^{13}\text{C}$ -enriched chlorosome preparations. The lines observed in  $^{13}\text{C}$ - $^{13}\text{C}$  correlation datasets were very narrow, of the order of 1 ppm (Figs. S1 and S2). A  $^{13}\text{C}$ - $^{13}\text{C}$  dataset recorded at a longer mixing time of 5.1 ms was needed to assign the farnesyl tail of BChl *d*, which meant that the tail is subject to restricted mobility. The assignments were made following previous NMR work on *C. tepidum* chlorosomes (15).

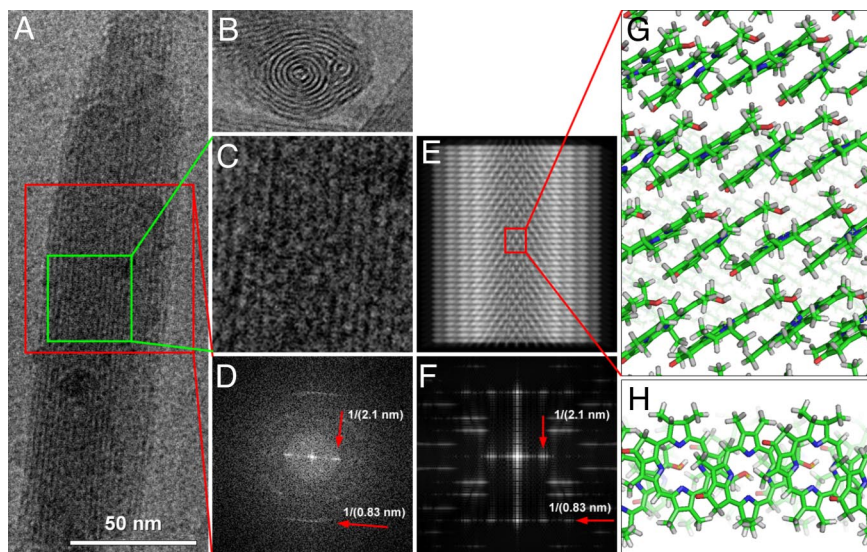
To proceed from the NMR chemical shift into the structure at the molecular level, proton-mediated correlation spectroscopy with a short mixing time of 250  $\mu$ s was used (Fig. 2*A*) (19). Intermolecular correlations between BChl *d* molecules within a chlorosome were detected for  $^1\text{H}$ - $^1\text{H}$  transfer events of  $<3$  Å (Fig. 2*A* and Table 1). Because no intramolecular pathway that could serve to correlate the resonances over a 250- $\mu$ s mixing time exists in the proton network, these correlations could be assigned unambiguously. The C-7/ $^1\text{C}$ -18, and C-7/ $^1\text{C}$ -18 $^1$  intermolecular contacts (Fig. 2*B*) observed were especially significant because very few possibilities for molecular assembly exist in which these atoms can be in close proximity. The data revealed the presence of alternating monomers that form stacks assembled into layers with the farnesyl tails alternately extending on both sides. The two possible intermolecular arrangements that could satisfy these constraints are the new stacking model based on alternating *syn* and *anti* monomers (Fig. 3*D*) or antiparallel monomer stacking (Fig. 3*C*).

In addition to the intrastack correlations, intermolecular correlations between carbons C-12<sup>1</sup>/C-3<sup>1</sup>, C-12<sup>1</sup>/C-2<sup>1</sup>, and C-12<sup>1</sup>/C-3<sup>2</sup> were detected between rings I and III of adjacent BChl *d* molecules. When *syn-anti* monomer stacks are combined to form a sheet, these distances are very short (Fig. 2C and Table 1), whereas for the antiparallel monomer stacking the shortest <sup>1</sup>H–<sup>1</sup>H distances between C-12<sup>1</sup>/C-3<sup>1</sup>, C-12<sup>1</sup>/C-2<sup>1</sup>, and C-12<sup>1</sup>/C-3<sup>2</sup> are 3.46, 6.28, and 2.20 Å, respectively. Because the short mixing time of 250 μs produces <sup>1</sup>H transfer over short distances <3 Å, the interstack correlations provide convincing evidence that *syn-anti* monomer stacking is the basic building block in the *bchQRU* chlorosome structure.

In chlorophyll aggregates, the  $^1\text{H}$  signals shift upfield by ring current effects from neighboring molecules (20). Density functional theory calculations were performed to estimate the ring currents for the *syn-anti* monomer model that was determined from the distance constraints, the antiparallel monomer model, and two earlier structural models that were proposed for BChl *c* in chlorosomes: the monomer-based, parallel-stack model (21) and the piggy-back dimer model (22) (Fig. 3). The *syn-anti* monomer stack has alter-







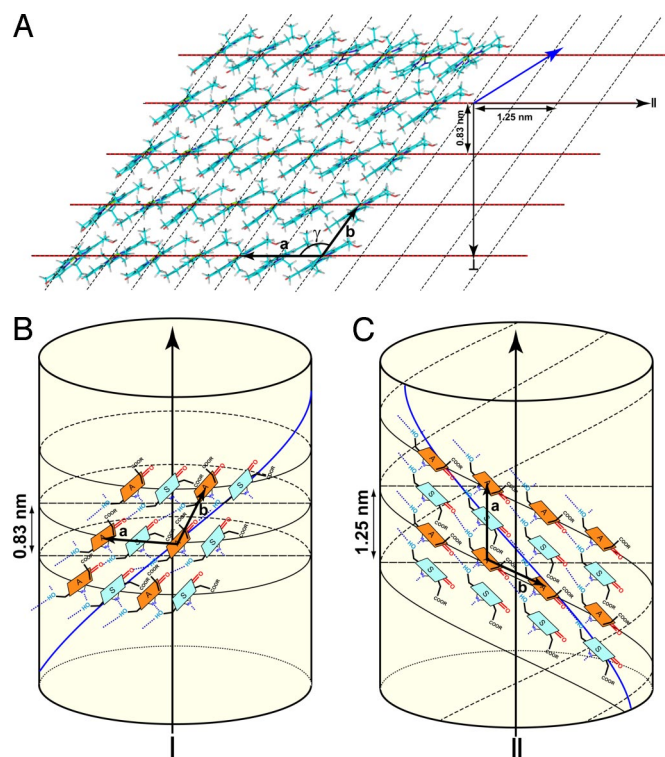
**Fig. 4.** Integration of cryoEM and NMR data to resolve the *bchQRU* mutant chlorosome structure. (A and B) CryoEM images of isolated chlorosomes from the *bchQRU* mutant of *C. tepidum* embedded in amorphous ice. A chlorosome is seen in side-on view (A) and in end-on view, showing the involuted layering of the BChls (B). (C) Enlarged region of the side-on view, indicated by the green box in A. (D) Fourier transform of the red-boxed region in A. The red arrows indicate reflections from a 2.1-nm spacing between BChl layers and a 0.83-nm spacing along the layers. (E and F) Simulated EM image and its Fourier transform, from a 17<sup>2</sup>-farnesyl-*R*-[8-ethyl, 12-methyl]BChl *d* helical rod model comprising 4 tubes built from NMR-derived and geometry-optimized *syn-anti* monomer stacks that run along the circumference of the tubes, perpendicular to their long axis. The 1/0.83-nm periodicity corresponds to the spacing between adjacent BChl stacks running perpendicular to the tube axis. As explained in the text, there are no additional layer lines for shallow helices, and the blurring of the 2.1-nm reflections on the equator is because of disorder. (G) Enlarged view of the boxed area in E. (H) Top view, i.e., along the tube axis.

Additionally, end views of WT chlorosomes by cryoEM had shown that the internal organization of BChls was unique in individual chlorosomes (18). This extensive heterogeneity had thus far prevented a precise determination of the structure at the molecular level. However, the chlorosomes of the *bchQRU* mutant provided the possibility for understanding the under-

pinning of the chlorosome suprastructure, optical properties, and variability among species.

The two supramolecular arrangements, with stacks running parallel or perpendicular to the tube axis, produce similar O—H...O=C exciton delocalization pathways. Both for *syn-anti* monomer stacks running perpendicular and for stacks parallel to the tube axis, the monomer transition moments align along the H-bond network to form helices along the concentric tube walls (Fig. 5 B and C). The optical transition dipole moment for the monomer runs through the C-13<sup>1</sup> carbonyl group, along the hydrogen bonds. After excitation, the excitons would move through the BChl aggregates along helical trajectories following the O—H...O=C connection pathway, which reflects the macroscopic chirality of the system (8). When tubes are formed by bending the molecular arrays in the opposite direction, the helicities also change sign. The bending direction will thus affect the circular dichroism sign, which has often been reported to vary in different preparations (23). The inclination of the optical transition dipole moment of the monomers with respect to the long axis of the tube in Fig. 5B is  $\approx 55^\circ$ . The structural arrangement of the BChls in sheets with overlap between the stacks allows for strong triplet-triplet exciton interactions, which forms the basis for the self-protection of the chlorosome against photodegradation in high light under oxic conditions (6).

Chlorosomes were probably an early invention in biology because they are found in three of the six groups of chlorophototrophs, and two of those three groups have homodimeric type 1 reaction centers (1, 2). The self-organization of the BChls into layers spaced by 2.1 nm is a common property that is preserved across species and allows for the inclusion of carotenoids and quinones by packing between the esterifying alcohols of the chlorins (18). The concentric tube structure of chlorosomes is optimal for very efficient energy transfer in a very large antenna, a prerequisite for photosynthetic growth under very weak light conditions. The suprastructure is stabilized by the underlying principles of the self-assembly process, whereas its size and heterogeneity can be optimized for optimal functionality. As measured by the growth rate of cells, the larger, more complex and heterogeneous WT chlorosomes outperform the chlorosomes of the *bchQRU* mutant at all light intensities between 5 and 200  $\mu\text{mol}$  of photons  $\text{m}^{-2} \text{s}^{-1}$  (12). Consistent with the idea that BChl *c* in a larger number of environments leads to better light harvesting, the half-band width of the  $Q_y$  absorption maximum of the BChl *c* aggregates is largest for WT chlorosomes isolated from cells grown at low light intensity and is also much broader than for chlorosomes of the *bchQRU* mutant (12). When grown at very low



**Fig. 5.** Structural models of chlorosomes in the *bchQRU* mutant and the WT. (A) 7  $\times$  5 section of the *syn-anti* array. The stacks are indicated by red lines. The separation perpendicular ( $\perp$ ) to the stacks is 0.83 nm. The unit cell dimensions are:  $a = 1.25$ ,  $b = 0.98$ ,  $\gamma = 122^\circ$ . The repeat in the direction parallel ( $\parallel$ ) to the stack is 1.25 nm. (B and C) Multistart helices with the long axis running perpendicular or parallel to the stacks. Periodicities of 1.25 nm and 0.83 nm are indicated. The *syn-anti* pairs in B form a multistart helix with rotational symmetry. The H-bond helices that connect BChl stacks via the carbonyls are shown in blue and are right-handed in B and left-handed in C.

light intensity ( $\approx 5 \mu\text{mol}$  of photons  $\text{m}^{-2} \text{s}^{-1}$ ), *C. tepidum* cells produce approximately three times more BChl *c* than cells grown at high light intensity ( $>100 \mu\text{mol}$  of photons  $\text{m}^{-2} \text{s}^{-1}$ ). Because the cell size does not change and the number of chlorosomes per cell does not change dramatically, the number of BChls per chlorosome must increase by  $\approx 3$ -fold (12).

Because the electronic properties of the supramolecular system and the absorption cross-section of the antenna can be modified by introducing disorder at the molecular level, functional advantages are achieved by adopting a suprastructure that is sufficiently plastic and flexible to accommodate considerable chemical heterogeneity. In the modeling, the two configurations having stacks running parallel or perpendicular to the tube axis correspond to local minima in the energy landscape of the suprastructure that permit chemical heterogeneity to be embedded within the structure. The layer lines observed in the Fourier transforms of the cryoEM data from the WT reveal extended periodic regions of stacks built from BChl pairs that run parallel to the tube axis in the suprastructure (Fig. 5C). Hence, the suprastructure is sufficiently stable to accept BChl *c* with methyl or ethyl side chains at C-12 and ethyl, propyl, or isobutyl side chains at C-8 of the tetrapyrrole ring. Likewise, the suprastructure must accommodate the variable amounts of BChls with *R*- and *S*-chirality at C-3<sup>1</sup> that the WT organism normally produces. This provides a window for evolutionary optimization through natural selection by diversification in the BChl side chains by coupling the replication/reproduction machinery (i.e., the growth rate) to the biosynthesis of the chlorophylls.

To couple non-protein-based light harvesting to gene evolution required the optimization of BChls and chlorosomes with respect to the predominant environmental constraint, low light intensity. Unmodified [8-ethyl,12-methyl]BChl *d*, found in the *bchQRU* mutant, with absorption similar to Chl *d*, is the easiest of the compounds to synthesize, requiring only three steps beyond chlorophyllide (12). However, its more regular, self-assembled structure has suboptimal optical properties. With essentially only 17<sup>2</sup>-farnesyl-[8-ethyl,12-methyl]BChl *d* the organism produces chlorosomes with very large BChl exciton domains and few nanotubes per chlorosome. The absorption spectrum is much narrower and is shifted to the blue by  $\approx 15$  nm compared with the WT, which still leaves significant overlap with the absorption of Chl *a* that is typically found in the overlying oxygenic aerobic chlorophototrophs (12). Three additional biosynthetic steps (six in total) are required beyond chlorophyllide to synthesize natural BChl *c*, and this probably has biological significance. The methylations at C-8 and C-12 in the WT contribute to tuning the wavelength of the  $Q_y$  absorption maximum and to increasing the absorption cross-section by inhomogeneous broadening because of the many different microenvironments of the BChls (12). In addition, *C. tepidum* synthesizes a higher proportion of BChls with *S*-chirality at C-3<sup>1</sup> at low light intensity (24), and this may produce a larger number of “BChl domains” per chlorosome as well as smaller “domains” as marked by edges. The robust structural framework allows the organism to avoid producing arrays with larger and larger numbers of BChl *c* at decreasing light intensity. A very large increase in the domain size of the antenna would probably reduce the energy transfer efficiency to the baseplate BChl *a* (B<sub>790</sub>), to the BChl *a*-binding Fenna–Matthews–Olson protein, and ultimately to the reaction centers.

Finally, the scaffolding according to Figs. 4 and 5 confirms that the structure of the chlorosome is established by self-assembly driven by noncovalent interactions without requiring proteins to provide coordinating ligands and structural control of BChl positioning. These interactions are (i) coordination of the C-3<sup>1</sup>-hydroxyl to the magnesium ion, (ii) hydrogen bonding between the C-3<sup>1</sup>-hydroxyl to the C-13<sup>1</sup>-keto group, (iii)  $\pi$ - $\pi$  stacking between the extended chlorin chromophores (20), (iv) electrostatic interactions, and (v) partitioning of aliphatic tails into the hydrophobic parts of the suprastructure. In particular the polarized keto group with negative charge on oxygen interacts electrostatically with the  $\text{Mg}^{2+}$ ,

which is considered the most important electrostatic contribution to the aggregation (2). Because it does not involve any direct binding of the keto moiety to the metal, it contributes to strengthening the hydrogen bond, in contrast to inferences from chemical modeling studies (5).

In conclusion, researchers have debated for decades about the structure of the chlorosome, which consists of hundreds of thousands of BChls self-assembled into the most efficient light-harvesting antennae in nature. By genetic modification of the BChl biosynthesis pathway, the inherent disorder in the chlorosomes of *C. tepidum* was reduced without functionally disturbing the self-assembly process. Computational integration of cryoEM and solid-state NMR was used in this work to extend and connect the structure information from the molecular to the supramolecular level for the *bchQRU* mutant to obtain very detailed structure information. This method may well prove useful in the future for the solution of other biological structures that cannot be crystallized or are inherently disordered. The mutant structure provides a template for the evaluation of the more complex structure of the WT. Both chlorosome suprastructures are built from *syn-anti* monomer stacks self-assembled into nanotubes. The basis for the efficient and ultrafast light harvesting is a helical exciton delocalization pathway that is realized with stacks running parallel or perpendicular to the tube axis in WT and mutant, respectively. The suprastructure can accommodate heterogeneity in the side chains for evolutionary optimization of light harvesting without the direct intervention of proteins to scaffold the pigment molecules. This latter feature must have been critical to the evolution of these structures in natural environments that are severely energy limited.

## Materials and Methods

**Sample Preparation.** Cells of the *C. tepidum bchQRU* triple mutant strain described by Gomez Maqueo Chew et al. (12) were grown as described by Balaban et al. (14). Samples of the corresponding WT strain were also prepared for comparison. Cultures were grown in 1.5-L fermentation bottles with continuous slow stirring at 40 °C. Growth light was controlled at the surface of the bottles ( $25 \mu\text{E m}^{-2} \text{s}^{-1}$  photosynthetically active radiation) provided by two 40-W fluorescent neon tubes. For the *bchQRU* mutant and the WT, cells uniformly labeled with <sup>13</sup>C were obtained in steps. First, a 50-mL inoculum of cells grown in Wahlund medium was inoculated into Wahlund medium without acetate (1 L). In the next step, a 50-mL cell inoculum obtained from the acetate-free culture was inoculated into Wahlund medium (1 L) containing sodium [<sup>13</sup>C]bicarbonate as the sole carbon source. Cells were grown for 3 days and then harvested by centrifugation. Chlorosomes from the two cell types, with the BChl *a*-containing baseplate attached, were isolated as described in ref. 14. Membranes were incubated for 1 h with 0.7% (wt/vol) lauryl dimethylamine oxide (LDAO) at an  $\text{OD}_{\text{max}} = 20 \text{ cm}^{-1}$  at the wavelength of maximum absorbance in the  $Q_y$  region between 700 and 750 nm. Chlorosomes were isolated by sucrose density gradient centrifugation; the gradients had a bottom layer of 50% (wt/vol) sucrose and a linear gradient from 10 to 40% (wt/vol) sucrose. The collected chlorosome band was diluted in 20 mM Tris-HCl (pH 8.0) to bring the sample below the critical micellar concentration ( $\approx 1$ –2 mM) of LDAO. The sample was washed to remove sucrose and LDAO by ultrafiltration in an Amicon concentrator equipped with a YM-100 membrane. The final chlorosome suspensions in Tris-HCl buffer showed the expected visible absorption maxima. Chlorosomes were kept in the dark at  $\approx 4$  °C before EM and NMR data collection. The pigments from the chlorosomes of the *bchQRU* mutant were also isolated and analyzed following the methods described in Steensgaard et al. (25), and  $>95\%$  of 17<sup>2</sup>-farnesyl-*R*-[8-ethyl,12-methyl]BChl *d* was found. For the EM, chlorosomes were also prepared with the NaSCN method (26). To facilitate the freezing process for EM, the 2 M NaSCN was removed by dialysis against buffer. The tendency of chlorosomes to clump together was much less with the NaSCN than for the LDAO preparation, which facilitated the EM measurements.

**NMR Measurements.** All solid-state, MAS NMR experiments were performed with a Bruker AV-750 spectrometer equipped with a 4-mm triple resonance MAS probe head (Bruker) by using a <sup>13</sup>C radio frequency of 188.6 MHz and a sample temperature of 277 K. Spinning frequencies of  $11 \text{ kHz} \pm 5 \text{ Hz}$  and  $13 \text{ kHz} \pm 5 \text{ Hz}$  were used for 2D <sup>13</sup>C-<sup>13</sup>C homo- and <sup>1</sup>H-<sup>13</sup>C heteronuclear correlation experiments, respectively. The <sup>1</sup>H spins were decoupled during acquisition by using the two-pulse phase modulation scheme (27) in all of the experiments. Two-dimensional <sup>13</sup>C-<sup>13</sup>C dipolar correlation spectra were recorded by using the radio



frequency-driven, dipolar recoupling sequence (28) with phase-sensitive detection in  $\omega_1$  at mixing times of 1.4 ms (Fig. S1), 2.9 ms (Fig. S2), and 5.1 ms. A  $^1\text{H}$   $\pi/2$  pulse length of 3.1  $\mu\text{s}$  was used with cross-polarization periods of 2 ms. For each of the 256 steps in the indirect dimension, 128 scans were collected. Two-dimensional  $^{13}\text{C}$ - $^{13}\text{C}$  spectra were recorded by using the CHHC/CP $^3$  sequence (19, 29) for indirect detection of  $^1\text{H}$ - $^1\text{H}$  contacts at  $^1\text{H}$  diffusion times of 250  $\mu\text{s}$  (Fig. 2A) and 325  $\mu\text{s}$ . The initial CP contact time was set to 256  $\mu\text{s}$ . Short CP contact times of 128  $\mu\text{s}$  enclosing the  $^1\text{H}$ - $^1\text{H}$  spin diffusion step were used to ensure that the polarization transfer was restricted to directly bonded  $^1\text{H}$ - $^{13}\text{C}$  spin pairs. For each of the 256 steps in the indirect dimension, 128 scans were collected. Two-dimensional  $^1\text{H}$ - $^{13}\text{C}$  heteronuclear correlation data were collected by using the frequency-switched Lee-Goldburg (FSLG) experiment (30), with a short CP time of 256  $\mu\text{s}$  and a  $^1\text{H}$  90° pulse of 3.1  $\mu\text{s}$  (Fig. S1). The  $^1\text{H}$  chemical shift scale was calibrated from a FSLG spectrum of solid tyrosine-HCl salt and validated with the chemical shift correlation plots (Fig. S6). For each of the 128 steps in the indirect  $^1\text{H}$  dimension, 128  $^{13}\text{C}$  scans were accumulated.

**CryoEM Measurements.** Aliquots of purified chlorosomes were applied to holey carbon grids with a thin layer of carbon and were plunge-frozen in liquid ethane at 83 K with a Vitrobot vitrification system (FEI). Electron microscopy was performed with a Tecnai G2 Polara electron microscope (FEI) equipped with a Gatan energy filter at 115,000 $\times$  magnification and a specimen temperature of 80 K. Images were recorded in the zero-loss imaging mode, by using a slit-width of 20 eV, with a slow-scan CCD camera at 1  $\mu\text{m}$  underfocus, to have optimal phase contrast transfer at 300 kV for details with a periodicity of  $\approx 2$  nm. Rotational averaging of EM data revealed concentric tubes (Fig. S7). EM images were

simulated as projected electron density from atomic coordinates at 0.6-nm resolution by using the EMAN pdb2mrc program (31) (Fig. 2).

**Structure Modeling and Ring Current Calculation.** A larger section of the *syn-anti* structure comprising of six stacks with 12 interacting BChl *d* molecules each was optimized in the computer by using an MM+ force field with a Polak-Ribiere conjugate gradient algorithm and with a gradient convergence criterion of 0.01 kcal/mol in Hyperchem 7. A *syn-anti* pair taken from this optimized structure was used to construct the larger multilayer tubular structures. From these atomic models, the electron density was calculated and projected down to create side views at a resolution of 0.6 nm for comparison with the experimental cryoEM data. In this way a composite image is produced by integration of the constraints obtained by the two methods, cryoEM and NMR, which can be visually compared with the biological specimen (Fig. 4). Density functional theory calculations were performed by using the Gaussian 03 software package (32) and the Becke, Lee, Yang, and Parr (BLYP) (33, 34) exchange-correlation functional, which has been used before to estimate NMR shifts for Chl systems (35). The 6-311++G(d,p) Gaussian basis set was used. To calculate the magnetic shielding caused by the local magnetic field induced by the ring current effect in the BChls, nucleus-independent chemical shift quantum mechanical calculations were performed (36). Ring current shifts were calculated by using the gauge-independent atomic orbital method (37-39).

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